Attorney Docket No.: 5573.200-US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Peschke et al.

Application No.: 09/337,809

Filed: June 21, 1999

Group Art Unit: 1653

Examiner: D. Lukton

For: Compounds with Growth Hormone Releasing Properties

DECLARATION UNDER 37 CFR 1.132

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I, Bernd Peschke, do hereby state and declare that

1. I am a citizen of Germany residing in Måløv, Denmark.

2. I have a doctorate in Chemistry and am currently a research chemist for Novo Nordisk A/S. I am also a co-inventor of the present invention.

3. I have read and am familiar with the present patent application and the Office Action dated April 4, 2000.

4. In support of the present application, the following experiments were performed.

5. For the in vivo characterization in conscious swine, one female 30 - 40 kg Danish slaughter swine of the breed Landrace Yorkshire cross was used for each GH secretagogue. The swine were housed at least one week prior to the experiment. Prior to the experiment, it was checked that the swine used had similar basal GH levels. Indwelling jugular catheters were inserted and fixed under general halothane anesthesia at day 0. The test compounds were administered as 50 nmol/kg iv bolus injections with at least 48 hour intervals between compounds. Each swine was used to test a maximum of five different compounds.

The test compounds were dissolved in phosphate/citrate buffer diluted in saline containing 0.5% porcine serum albumin. Blood samples were drawn from the jugularis catheter at frequent intervals from 1 hour prior to stimulation until 3 hours post stimulation.

6. The compounds described in Examples 1, 6, 7, 10, 11, 12, 18, 20 and 23 were tested in the experiment described above. All compounds are active in vivo as growth hormone secretagogues. As demonstrated in the table below, the compounds described in Examples 10 and 18 show high activity, compounds described in Examples 1, 6, 7, 12, 20 and 23 show moderate activity, and the compound described in Example 11 shows weak activity. This data shows that the compounds described in the above-referenced patent application are active in vivo as growth hormone secretagogues.

Example No.	In vivo GH-release in swine (ng/ml)
1	20
6	24
10	53
11	9
12	19
18	20
20	15
23	14

7. For the compound described in Example 15 a different experiment was performed. Female slaughter pigs (Danish Landrace) with an initial weight of 40-45 kg at the time of first dosing were used. The animals were allowed a one-week acclimatization period before blood sampling catheters (vena jugularis & arteria carotis) and an intra gastric (dosing catheter) were inserted under general anesthesia (Propofol / Isoflurane). The animals were then allowed to recover at least 5 days before they were studied. A Latin square design was used in all experiments. In this design all animals receive different test substances in an experiment series once, but in a different order to allow for all possible combinations of test substances. A minimum 2-day washout period is placed between the dosings to minimize the possible risk of a carry-over effect. This design makes best possible use of the

experimental animals and circumvents the very time consuming task of surgical catheter implantation without losing power with respect to the number of animals per test substance.

In a typical experiment series, approx. 3.5 mg/kg (0.005 mmol/kg) of the compound was dissolved in sterile water and was dosed absolutely stress free directly into the gastric lumen via the dosing catheter. Blood samples were taken at time intervals –30, -15, 0, 5, 15, 30, 45, 60, 120, 180, 240 minutes relative to the time of dosing.

Statistical analysis, calculation of computed parameters such as Area under the Curve ($Hormone_{AUC}$) and time to maximum concentration ($T_{max}Hormone$) as well as identification of maximum hormone concentrations ($C_{max}Hormone$) were done using the SAS (v6.12) statistics package.

8. As demonstrated in the table below, the compound of Example 15 is a very potent growth hormone secretagogue in vivo. It is almost equipotent to the most potent orally active growth hormone secretagogue known at the time the present application was filed, namely, N-[1(R)-[(1,2-Dihydro-1-methanesulfonylspiro[3H-Indole-3,4'-piperidine]-1-4-yl)carbonyl]-2-(phenylmethyloxy)-ethyl]-2-amino-2-methylpropanamide (MK-0677). And it is more potent than 5-Amino-5-methylhex-2-enoic acid N-methyl-N-((1R)-1-(N-methyl-N-((1R)-1-(methylcarbamoyl)-2-phenylethyl)carbamoyl)-2-(2-naphthyl)ethyl)amide (NN-703) disclosed in U.S. Patent No. 5,977,178.

Test Compound	Cmax (ng/ml)
Example 15	38
MK-677	44
NN-703	18

9. The undersigned declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize any patent issuing thereon.

Signed this 22 day

of September 2000

Bernd Peschke

Bernd Peschke

Attorney Docket No.: 5573.200-US

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In re Application of: Peschke et al.

Application No.: 09/337,809 Group Art Unit: 1653

Filed: June 21, 1999 Examiner: D. Lukton

Confirmation No: 9496

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Assistant Commissioner for Patents
Washington, DC 20231

Sir:

I, Bernd Peschke, do hereby state and declare that

1. I am a citizen of Germany residing in Måløv, Denmark.

2. I have a doctorate in Chemistry and am currently a research chemist for Novo Nordisk A/S. I am also a co-inventor of the present invention.

3. I have read and am familiar with the present patent application and the Office Action dated May 7, 2002 and understand that the Examiner would like a showing of the baseline growth hormone level.

4. In support of the present application, the following experiments were performed.

5. Female Danish slaughter pigs were ordered with a weight range between 35 and 40 kg live weight at time of delivery. The pigs had a minimum of 2 weeks to acclimatize to their new environment before they were implanted with catheters. Pigs had a minimum of 3 days to recover from surgery before they were dosed with compound.

6. Stomach catheters were inserted into the pigs using the following procedure.

a. Each pig was fasted for 24 hours before surgery, but had free access to water. On the day of surgery, the animals were anaesthetised with a mixture of: Zoletil, Xylazin, Ketaminol and Methadone. 1ml/15kg BW of this mixture was given i.m. to induce anaesthesia within 5min after injection. Atropin (0.05 mg/kg) i.m. was given with the above mixture. Once the animal had lost consciousness, an ear vein catheter was inserted to gain venous access. A standard dose of antibiotic (Novocilin) was given i.v. at that time. The flank and the neck of the animal were then carefully clipped and shaved outside the operating theatre. The shaved areas were thoroughly disinfected with chlorhexidine and iodine solution. All surgical procedures were performed under strictly aseptic conditions.

b. The animal was positioned on the operating table and an endotracheal tube was inserted. Total relaxation of the pig was induced by administration of a 5-10ml Propofol (5mg/kg) (Rapinovet) bolus. After intubation, anaesthesia was maintained by 1-1.5% isoflurane in 2 l/min 100% oxygen. Each pig was kept on spontaneous respiration. All vital data were noted on the anaesthesia protocol. The pig was also connected to a saline drip infusion (500 ml/h) to stabilise its cardio-vascular system.

c. A laparotomy was performed with the pig in left recumbency. The stomach was gently retracted and a purse-string suture was placed in the muscular part of the stomach wall 5-8 cm from the pyloric end. A hole was made with a diatherimic needle and a silicone catheter was inserted into the gastric cavity. The catheter was held in place by 2 silicone cuffs, one inside and one outside the stomach wall. The wound was closed using standard techniques and the catheter was exteriorised at the back of the animal. The pig was then placed in dorsal recumbency and a paramedian skin incision was made over the jugular furrow. The jugular vein and carotid artery were dissected and fitted on 20 cm with a silicone catheter. The catheters were tunnelled to the back of the neck and the wound was closed using standard techniques.

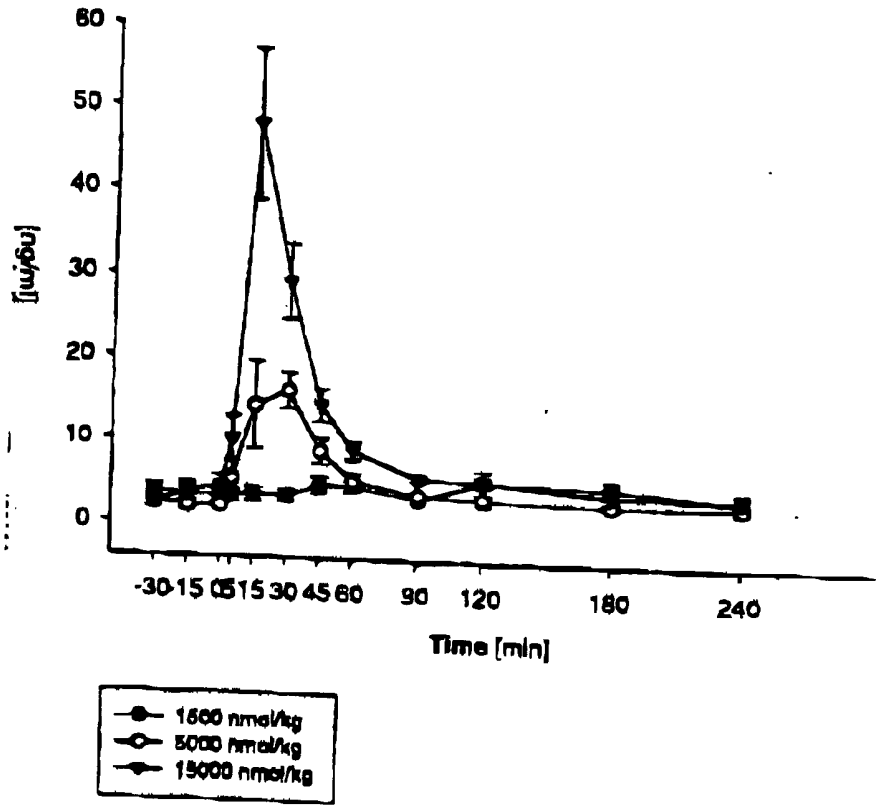
d. All animals received a standard 4 day course of antibiotic (Novocillin). An analgesic (Fenadyne, 0.5 ml/10kg BW, i.v.) was administered for 3 days after surgery. All blood sampling catheters were flushed daily with 10 ml of saline for the first 3 days and thereafter every third day.

7. The following procedure was used to administer the compound of Example 15, the acetate salt of (2E)-4-(1-aminocyclobutyl)but-2-enoic acid N-((1R)-1-{N-[(1R)-1-benzyl-2-(4-hydroxypiperidin-1-yl)-2-oxoethyl]-N-methylcarbamoyl}-2-(biphenyl-4-yl)ethyl)-N-methylamide]. Pigs were dosed orally at 09:00 hours after an overnight fast. The acetate salt of the compound of Example 1 was freshly dissolved in sterile water to the required concentration and dosed as a 20 ml bolus. The stomach catheters were flushed with 150 ml water for injection. Blood samples (3.5 ml) were taken at -30, -15, 0, 5, 15, 30, 45, 60, 90, 120, 180 and 240 min relative to the time of dosing of the animals. The blood was transferred to tubes containing EDTA, placed on ice and centrifuged at least once every 60 min (3000 rpm, 10 min, 4°C). Plasma was frozen at -20°C until analysis. The pigs were dosed with increasing doses (1500, 5000, 15,000 nmol/kg BW) of the compound of claim 1 with a 48h wash-out

period between doses. Plasma samples were analysed for growth hormone by radio-immunoassay, fully validated for porcine plasma.

8. The results are shown in the figure below:

Growth hormone response to increasing doses of Example 15
Pigs were dosed p.o. at time 0. Data are mean±SEM.



9. The results indicate that administration of the compound of Example 15, which is encompassed by the claims of the instant application, increases growth hormone levels in a dose-dependent manner within 15 minutes

of administration. Clearly, the growth hormone level is significantly higher than baseline after administration of 5000 nmol/kg and 15000 nmol/kg of the compound of Example 15.

10. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: November 5, 2002

Bernd Peschke

Bernd Peschke